*Journal of Chemical Ecology, Vol. 18, No. 3, 1992*

# ELECTROANTENNOGRAM RESPONSE OF ALFALFA SEED CHALCID, *Bruchophagus roddi* (HYMENOPTERA: EURYTOMIDAE) TO HOST- AND NONHOST-PLANT VOLATILES

DOUGLAS M. LIGHT,[1,*] JAMES A. KAMM,[2] and RONALD G. BUTTERY[1]

[1]*USDA-ARS Western Regional Research Center*
*800 Buchanan Street*
*Albany, California 94710*

[2]*USDA-ARS National Forage Seed Production Research Center*
*3450 S.W. Campus Way*
*Oregon State University*
*Corvallis, Oregon 97331*

(Received June 20, 1991; accepted October 28, 1991)

**Abstract**—The alfalfa seed chalcid (ASC), *Bruchophagus roddi*, is a monophagous pest of alfalfa that parasitizes developing seeds. To further understand the olfactory basis of host-plant recognition by ASCs, we recorded electroantennograms (EAGs) from females and males to 39 volatiles from both alfalfa and red clover. The chemoreceptive sensitivity of ASCs was selective for certain general classes of compounds, defined by their carbon-chain length ($C_6$ and $C_8$), structure (aliphatics and phenolics), isomerism, and/or functional group (acetates, ketones, and alcohols). The compounds that elicited the largest EAGs were ranked as follows: (Z)-3-hexenyl acetate > hexyl acetate ≥ acetophenone ≥ octan-3-one ≥ methyl salicylate > octan-3-ol > oct-1-en-3-one > oct-1-en-3-ol ≥ (*E*)-β-ocimene ≥ (*Z*)- and (*E*)-3-hexen-1-ols. Over half the test compounds elicited significantly different responses between the sexes, and female antennal responses exceeded those of males for twice the number of these volatiles. Relationships of the tested volatiles to host-plant composition, EAG responses, and ASC behaviors showed no consistent correlations. However, nearly all of the host-plant volatiles known to stimulate behavioral activity also elicited moderate to potent EAG responses.

**Key Words**—Alfalfa seed chalcid, *Bruchophagus roddi*, Hymenoptera, Eur-

*To whom correspondence should be addressed.

ytomidae, alfalfa volatiles, plant odors, electroantennogram, chemoreception, olfaction, kairomones, host plant selection, orientation, oviposition.

## INTRODUCTION

The alfalfa seed chalcid, *Bruchophagus roddi* (Gussakovsky) is a monophagous pest of alfalfa (*Medicago sativa* L.). Alfalfa seed chalcids (ASC) will oviposit only in alfalfa seed pods and not in pods of red clover (*Trifolium pratense* L.) or other sympatric forage legumes (Strong, 1962). When ASC females were exposed to the odors of alfalfa and red clover (either whole plant parts or extracts) in a flight-tunnel bioassay arena, they flew to, landed on, and explored targets emanating alfalfa but not red clover odors (Kamm and Buttery, 1986a; Kamm, 1989). Moreover, when female ASCs were exposed to individual volatile components of alfalfa, they oriented to, alighted on, and explored targets impregnated with certain specific components, some of which also stimulated oviposition (Kamm and Buttery, 1983, 1986b).

More than half the volatile components in the odors of alfalfa and red clover are common to both species (Kami, 1978, 1983; Buttery and Kamm, 1980; Buttery et al., 1982, 1984; Srinivas, 1986) (Table 1). Hence, host-plant recognition based on olfaction by ASCs is a complex task of both sensory reception and integration. An array of relationships between plant volatile production by these two legumes and associated chalcid behaviors have been identified for ASC females (Kamm and Buttery, 1983, 1986b) (Table 1). Certain plant-specific volatiles have chalcid species-specific semiochemical activity (Kamm and Buttery, 1986a,b).

To further understand the olfactory basis of host-plant detection, we determined the chemoreceptive responsiveness and sensitivity of antennae of female and male ASCs, by recording the electroantennogram (EAG) responses to both alfalfa and red clover volatiles. The EAG responses of ASCs were then correlated to the composition of the plant emissions and the behaviors evoked by these volatiles, as determined in previous laboratory bioassays.

## METHODS AND MATERIALS

*Insects.* Test insects emerged in the laboratory from infested alfalfa seeds from Ontario, Oregon, as previously described (Kamm and Buttery, 1983). Chalcids were collected on the day of emergence and put into vials (95 × 24 mm) segregated by gender, provisioned with 10% sucrose water solution, and exposed to a photoperiod regime of 16 hr light–8 hr dark, at 24°C for 48–72 hr before a test.

*Olfactory Stimuli.* Table 1 lists the 40 test compounds (36 legume volatiles

TABLE 1. COMPOSITIONAL PERCENTAGE OF VOLATILES IDENTIFIED IN HEADSPACE ODORS OF PLANT PARTS OF ALFALFA AND A NONHOST PLANT, RED CLOVER,[a] BEHAVIORAL RESPONSE THRESHOLDS (IN $\mu g$ AND $\mu g/\mu l$) AND POTENCY RANKS OF THESE VOLATILES, WHEN PRESENTED SINGLY TO ELICIT FLIGHT AND PEDESTRIAN ORIENTATIONS, ARRESTMENT, AND OVIPOSITION RESPONSES FROM FEMALE ALFALFA SEED CHALCIDS IN PREVIOUS LABORATORY OLFACTOMETER STUDIES,[b] AND EXPERIMENTAL RANKS OF THESE COMPOUNDS AS TO THEIR POTENCY IN ELICITING ELECTROANTENNOGRAM RESPONSES FROM ALFALFA SEED CHALCIDS

| | Relative percent of headspace volatiles | | | | | | Olfactometer behavioral thresholds of female ASCs | | | | | | | Electroantennogram data analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Alfalfa | | | Red clover | | | Orientation | | | Oviposition | | Volatility indexes: | | Male ASCs ranking | | Female ASCs ranking | |
| Compounds in headspace trappings | Leaves | Flowers | Pods | Leaves | Flowers | Pods | Flight ($\mu g$: rank) | Walking ($\mu g$: rank) | Arrestment ($\mu g$: rank) | Stimulation ($\mu g/\mu l$: rank) | Inhibition ($\mu g/\mu l$: rank) | Mol. wt. (g/mole) | R. index /subset[c] | 10 $\mu g$ | 100 $\mu g$ | 10 $\mu g$ | 100 $\mu g$ |
| Aliphatic compounds | | | | | | | | | | | | | | | | | |
| Acetic acid[d] | | | | | | 3.0 | [g] | | | | | 60.1 | 580 A | 27 | 27 | 34 | 24 |
| Pent-1-en-3-one | | | | | | 4.0 | | | | | | 84.1 | 655 A | 24 | 18 | 35 | 10 |
| 2-Methylbutanol | | | | | | 4.0 | NR[h] | NR | NR | | NR | 88.2 | 718 AB | 32 | 17 | 38 | 22 |
| 3-Methylbutanol[e] | 0.5 | 7.0 | 3.5[i] | | | | NR | NR | NR | | NR | 88.2 | 714 AB | 28 | 16 | 33 | 9 |
| 2-Hydroxybutan-3-one | | | | | 3.0 | 3.0 | | | | | | 88.2 | AB | 38 | | 27 | |
| 2,3-Dihydroxybutane | | | | | 2.0 | | NR | NR | NR | | | 90.2 | AB | 30 | | 31 | |
| Hexan-2-one | | | | | 0.2 | | | | | | | 100.2 | 761 ABC | 35 | 26 | 30 | 20 |
| Hexanal | | | 2.0 | | | | NR | NR | NR | | NR | 100.2 | 722 ABC | 26 | 11 | 36 | 8 |
| (*E*)-2-Hexenal[f] | 0.2 | 0.5 | 1.3 | | 6.0 | 3.0 | 25.0: 10 | NR | NR | | NR | 98.2 | 822 BC | 20 | 1 | 26 | 28 |
| Hexan-1-ol | | | 0.5 | 0.5 | 0.7 | | | | | | | 102.2 | 848 BC | 10 | 9 | 17 | 14 |
| (*Z*)-3-Hexen-1-ol | 7.0 | 4.5 | 25.0 | 24.0 | 3.0 | | 250.0: 12 | 250.0: 12 | 2.5: 4 | | NR | 100.2 | 834 BC | 8 | 13 | 9 | 15 |
| (*E*)-2-Hexen-1-ol | | | 4.0 | 4.0 | 0.4 | | 0.25: 7 | 2.5: 9 | NR | | NR | 100.2 | 845 BC | 9 | 10 | 13 | 7 |
| Hexyl acetate | 0.2 | | | | | | 25.0: 6 | 25.0: 7 | 25.0: 5 | 0.1: 1 | | 144.2 | 995 C | 2 | 4 | 7 | 2 |
| (*Z*)-3-Hexenyl acetate | 78.5 | 8.1 | 14.5 | 33.0 | 3.0 | 2.0 | 250.0: 11 | 2.5: 6 | 2.5: 6 | 0.1: 3 | | 142.2 | 986 C | 1 | 8 | 1 | 1 |
| Heptan-2-one | | | | | 0.7 | | NR | NR | NR | | | 114.2 | 865 BC | 23 | 3 | 19 | 13 |
| Octan-3-one | <0.1 | 1.0 | 1.5 | | | | 0.025: 1 | 0.025: 2 | 0.025: 1 | 0.01: 4 | | 128.2 | 964 C | 7 | 6 | 2 | 17 |
| Oct-1-en-3-ol | 0.4 | 0.5 | 6.0 | 0.8 | 3.0 | 4.0 | 2.5: 8 | NR | NR | | NR | 128.2 | 962 C | 12 | 7 | 8 | 3 |
| Decyl acetate | | 7.0 | | | | 3.0 | | | | | | 200.3 | 1393 D | 14 | 20 | 25 | 25 |
| Dodecyl acetate | | 4.0 | | | | 3.0 | | | | | | 228.1 | D | 19 | 21 | 29 | 26 |
| Analogs | | | | | | | | | | | | | | | | | |
| Oct-1-en-3-one | | | | | | | | | | | NR | 126.2 | 953 C | 13 | | 5 | |
| Octan-3-ol | | | | | | | | | | | 0.1: 2 | 130.2 | 980 C | 5 | | 6 | |
| Monoterpenoids | | | | | | | | | | | | | | | | | |
| Myrcene | 1.1 | 3.0 | | | | | NR | 25.0: 11 | NR | 0.1: 2 | | 136.2 | 981 C | 33 | 28 | 32 | 29 |
| Limonene | | 0.5 | | | | | | | | | | 136.2 | 1020 C | 21 | 12 | 16 | 6 |
| (*Z*)-$\beta$-Ocimene | | | | 5.0 | 3.0 | 6.0 | NR | NR | NR | | | 136.2 | 1026 C | 17 | 15 | 14 | 19 |
| (*E*)-$\beta$-Ocimene | 1.9 | 25.0 | 4.0 | 22.0 | 8.0 | 35.0 | NR | NR | NR | | NR | 136.2 | 1037 C | 6 | 14 | 10 | 4 |

TABLE 1. Continued

| Compounds in headspace trappings | Relative percent of headspace volatiles[a] | | | | | | Olfactometer behavioral thresholds of female ASCs | | | | | Volatility indexes: | | Electroantennogram data analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Alfalfa | | | Red clover | | | Orientation | | | Oviposition | | | | Male ASCs ranking | | Female ASCs ranking | |
| | Leaves | Flowers | Pods | Leaves | Flowers | Pods | Flight ($\mu$g: rank) | Walking ($\mu$g: rank) | Arrestment ($\mu$g: rank) | Stimulation ($\mu$g/$\mu$l: rank) | Inhibition ($\mu$g/$\mu$l: rank) | Mol. wt. (g/mole) | R. index /subset[c] | 10 $\mu$g | 100 $\mu$g | 10 $\mu$g | 100 $\mu$g |
| Monoterpenoids | | | | | | | | | | | | | | | | | |
| Neryl 2-methylbutrate | | 1.0 | | | | | NR | NR | NR | | | 238.2 | D | | | 24 | |
| Analog: Linalool | | | | | | | NR | NR | NR | | | 154.3 | 1083 C | 11 | 19 | 12 | 11 |
| Aromatic compounds | | | | | | | | | | | | | | | | | |
| Benzyl alcohol | 0.4 | 0.6 | 5.0 | | | | NR | NR | NR | | NR | 108.2 | 1004 C | 27 | | 22 | |
| Acetophenone | | | | | 24.0 | 4.0 | NR | NR | NR | | | 120.2 | 1031 C | 4 | 5 | 4 | 5 |
| 1-Phenylethanol | | | | | 8.0 | | NR | NR | NR | | | 122.2 | 1031 C | 22 | 23 | 18 | 18 |
| 2-Phenylethanol | 0.3 | 1.0 | 5.5 | | 1.0 | | NR | 25.0: 10 | 25.0: 7 | | 1.0: 3 | 122.2 | 1081 C | 15 | 25 | 15 | 23 |
| Methyl salicylate | 0.8 | 2.5 | 1.5 | 0.4 | | | NR | NR | NR | | NR | 152.1 | 1166 C | 3 | 2 | 3 | 12 |
| Methyl cinnamate | | | | | 11.0 | | | | | | | 162.2 | 1348 D | 25 | | 11 | |
| Sesquiterpenes | | | | | | | | | | | | | | | | | |
| $\alpha$-Copaene[j] | 0.1 | 1.4 | 0.3 | | | | 2.5: 5 | 2.5: 8 | NR | | NR | 204.4 | 1373 D | 37 | | 23 | |
| Longifolene | | | | | | 5.0 | NR | NR | NR | | | 204.4 | 1402 D | 31 | 29 | 37 | 27 |
| Caryophyllene | <0.1 | 0.2 | 0.5 | 0.1 | 0.4 | 3.0 | 25.0: 9 | 2.5: 4 | 2.5: 3 | 10.0: 6 | | 204.4 | 1414 D | 16 | 22 | 20 | 16 |
| ($E$)-$\beta$-Farnesene | 0.1 | 0.1 | 4.0 | 2.0 | | 2.0 | 0.025: 2 | 0.025: 1 | NR | 1.0: 5 | | 204.4 | 1447 D | 18 | 24 | 21 | 21 |
| $\gamma$-Muurolene | 0.3 | 0.7 | 5.5 | | | | 2.5: 4 | 2.5: 5 | NR | | 0.1: 1 | 204.4 | 1469 D | 29 | | 39 | |
| Analog: Farnesol | | | | | | | 0.025: 3 | 0.025: 3 | 0.025: 2 | | NR | 222.4 | 1675 E | 34 | | 28 | |
| Total percentage: | 92.0 | 68.6 | 87.3 | 91.8 | 77.4 | 84.0 | | | | | | | | | | | |
| Number of compounds | 16 | 20 | 18 | 10 | 17 | 15 | 28: 12 | 28: 12 | 28: 7 | 22: 6 | 22: 3 | 39 | | 38 | 29 | 39 | 29 |

[a] The headspace volatiles in the atmosphere associated with the plant's intact leaves, flowers, and pods were adsorbed on Tenax substrates, extracted in back-washes of ether, concentrated, and then identified by comparisons of the complete mass spectrum and Kovats' GLC retention index of authentic samples, followed by GLC/FID integrative analysis of the percent relative occurrence in the trapped plant-odor; alfalfa analysis (Buttery et al., 1982) and Red clover analysis (Buttery et al., 1984).

[b] Kamm and Buttery (1983, 1986a, b).

[c] Retention index on a nonpolar DB-1 column (a bonded, cross-linked polymethylsiloxane coated fused-silica capillary column); this retention index is an approximate reflection of the volatility of a component relative to a scale of normal saturated, aliphatic hydrocarbons, where the assigned values are: e.g., butane = 400, pentane = 500, hexane = 600, etc.

[d] Compounds fully indented were found only in red clover analyses.

[e] Compounds not indented were found only in alfalfa analyses.

[f] Compounds partially indented were found in analyses of both plant species.

[g] Blank spaces represent the compound having not been found or tested.

[h] NR: no response elicited behaviorally by the compound.

[i] For the alfalfa composition analysis the 2- and 3-isomers of methylbutanol were not distinguished.

[j] The EAG responses reported for $\alpha$-copaene are an average of those recorded to separate samples of the (+) and (−) enantiomers.

and four analog compounds), their presence in different plant parts of the two legume species, and the type and threshold of behavioral responses of females when exposed to individual compounds in laboratory bioassays. Test chemicals were either obtained from commercial sources, synthesized, or isolated from plant extracts. All compounds were repurified by preparative gas chromotography separation and their identities verified by mass and infrared spectrometries. Purity was >95% for all compounds except the sesquiterpenes. The sesquiterpenes are not commercially available and therefore were separated from hop oils (Buttery et al., 1982) with the following purities: α-copaene (>81%), caryophyllene (>85%), (*E*)-β-farnesene (>93%), and γ-muurolene (>80%).

Test compounds were dissolved in spectrometric grade hexane to form volume-to-volume dilutions resulting in 10% and 1% solutions of compounds. For nine of the compounds the dilution series were taken to the 0.0001% or 0.00001% levels. For each test, 1 μl of a compound solution was pipetted onto a 1 × 2-cm piece of folded glass-fiber, Whatman No. 1 filter paper. For the 10% and 1% solutions approximately 100 μg and 10 μg, respectively, of test compound were placed on separate filter-paper odor sources. For the dose–response tests the dilution series spanned from 100 μg to 1 ng of test compound on the filter papers. Due to insufficient amounts of octan-3-ol and oct-1-en-3-one, their dilution series had to span from 10 μg to 0.1 ng. After the solvent was allowed to evaporate for 10 sec, the filter paper was inserted into a glass "test cartridge" (a disposable Pasteur pipet), which was then immediately attached to the test odorant delivery system. Each test cartridge (including the control and standard, see following) was prepared just prior to its testing, tested, and then discarded. Compounds were tested in random order for each chalcid replication. All solutions were stored at −50°C.

*Electrophysiological Recording Technique.* The electroantennogram procedure utilized glass capillary Ag–AgCl electrodes filled with insect saline, as described by Light et al. (1988). Test insects were immobilized by imbedding their dorsal side into low-melting-point wax affixed to one end of brass rod (3 × 100 mm). Antennae were secured with a strand of aramid fiber (Kevlar, DuPont DeNemours Co., Wilmington, Delaware). Under 128× microscope magnification, the brass rod mount was then positioned for ease of insertion of the recording electrode into the distal antennal tip. The indifferent electrode was inserted into the frons near the base of the scape. The signal was amplified 1000× by a microelectrode amplifier (P-16, Grass Instruments, Inc., Quincy, Massachusetts) and viewed on a digital storage oscilloscope (310, Nicolet Instruments, Inc., Madison, Wisconsin). EAG deflections were measured directly from the screen image and stored on floppy diskettes for later retrieval.

*Odor Delivery.* The odor delivery system was similar to that previously described (Light et al., 1988). Briefly, charcoal-filtered and humidified compressed air, delivered via Teflon tubing, was continuously passed over the

antenna at the rate of 1 liter/min through a disposable (pipet tip) nozzle positioned 1 cm from the antenna. Activation of a three-way solenoid valve diverted the purified air through a test cartridge where the evaporated volatiles were purged by the airflow and carried onto the antenna. The duration of an odor stimulation was 1.0 sec, as controlled by a precision time-delay relay circuit.

*Experimental Procedure.* The following experiments were conducted on ASC antennae: (1) 39–40 different volatiles at 10-$\mu$g dose, (2) 29 of those volatiles at 100 $\mu$g dose, and (3) nine of the volatiles (determined to be the most potent in the 10-$\mu$g experiment and/or prior behavioral bioassays) over a six-decade step series of doses. All three experiments were conducted on females, while only the two set-dose experiments were conducted on males. At least 3 min elapsed between stimulations to allow for adequate receptor recovery of ASC antennae. In the dose–response experiments compounds were randomly chosen for testing and then test doses for each compound were presented sequentially from lowest to highest. Different cohorts of individual chalcids were used for the three separate experiments. Each experiment was replicated on five different individuals per sex.

Interspersed between every fifth test odor stimulation were "control" stimulations (odor cartridges with filter papers impregnated with 1 $\mu$l of hexane solvent) and "standard" stimulations [odor cartridges with filter papers impregnated with 1 $\mu$l of a 1% solution of hexyl acetate (a 10-$\mu$g dose)]. For each individual chalcid, EAG responses were evaluated by measuring the maximum amplitude of the negative polarity deflection ($-$mV) elicited by each test volatile stimulation, minus the amplitude of the response to the accompanying solvent control. Then these "control-corrected" $-$mV responses to the test volatiles were converted for each individual to percentage values of the response to the accompanying 10-$\mu$g hexyl acetate standard. This normalization of each response to a percentage of a standard response permits comparison of responses within and between preparations (Payne, 1975).

*Data Analysis.* For the two set-dose experiments, statistical differences between the evoked EAG responses to the ensemble of legume volatiles were evaluated by ANOVA followed by Duncan's multiple-range test. Certain large differences between means were evaluated by paired $t$ test statistics for the dose–response data. Statistical differences between the sexes in their EAG response magnitudes for a set volatile at a set test dose were evaluated by $t$ test statistics.

Analysis of antennal sensitivity for the nine dose–response test compounds was based on three dose–EAG response curve parameters: threshold dose, maximum response, and the slope of the curve over its dynamic response phase (Light, 1983). The "threshold dose" is defined as the interpolated dose of a test compound that elicited an EAG response that just exceeded the average response to control stimulations (delineated by the horizontal zero lines in Figure 3 below). The "maximum EAG response" was elicited usually to the highest

(100 $\mu$g) dose of compound tested. The curve slope was calculated for the exponential "dynamic response phase" of each curve, when the rate of change in response is the greatest (i.e., steepest slope). Thus, higher antennal sensitivity to a compound is represented by lower threshold, greater slope, and greater maximum percent response values.

## RESULTS

### *Antennal Responses to Control and Standard*

The mean $-$mV responses of both female and male antennae to the solvent control stimulations were $< -0.20$ mV and uniform. The mean $-$mV responses to the standard stimulations (10 $\mu$g hexyl acetate) for both sexes were of a moderate magnitude. No significant difference in EAGs to standard was found between males ($X = -0.84$ mV $\pm$ 0.02 SEM, $N = 10$) and females ($X = -0.80$ mV $\pm$ 0.02 SEM, $N = 15$). However, a great degree of sexual dimorphism in response to many of the 38 legume volatiles was evident, with significant differences between the sexes found in response to 58% of the volatiles tested at the two set-doses.

### *Antennal Responsiveness*

*Limitations in Comparative Analyses.* Most test volatiles in Table 1 and Figures 1 and 2 are arranged by increasing molecular weight and GC retention index (nonpolar capillary column). The relative volatility of these compounds is generally correlated to these two physical property indexes. Thus, the relative number of molecules of a test volatile delivered to the antennae in these EAG tests varied tremendously over the full range of volatiles tested, from the three- and four-carbon aliphatic compounds to the 15-carbon sesquiterpenes.

Therefore, we have limited direct comparisons in EAG responsiveness to subsets of compounds, each subset comprised of compounds possessing a common narrow range in volatility, as delineated in Table 2 by a common capital letter following the retention time index values. Thus, EAGs to volatiles in the subsets at the extremes of volatility (i.e., the $\leq C_5$ volatiles and the sesquiterpenes) can be compared validly only with members of their subset. The EAGs elicited by the $C_6$ acetates (e.g., the experimental standard) can be compared to $C_7$–$C_8$ volatiles, monoterpenes, and most aromatic volatiles. However, valid relative comparisons in potency of EAGs are possible between compounds in different volatility subsets, if the compound with a lower volatility (i.e., far fewer numbers of molecules per stimulation puff) elicited EAG responses of equal or greater magnitude than those elicited by the compound with a higher volatility (i.e., far greater numbers of molecules per stimulation puff). One may

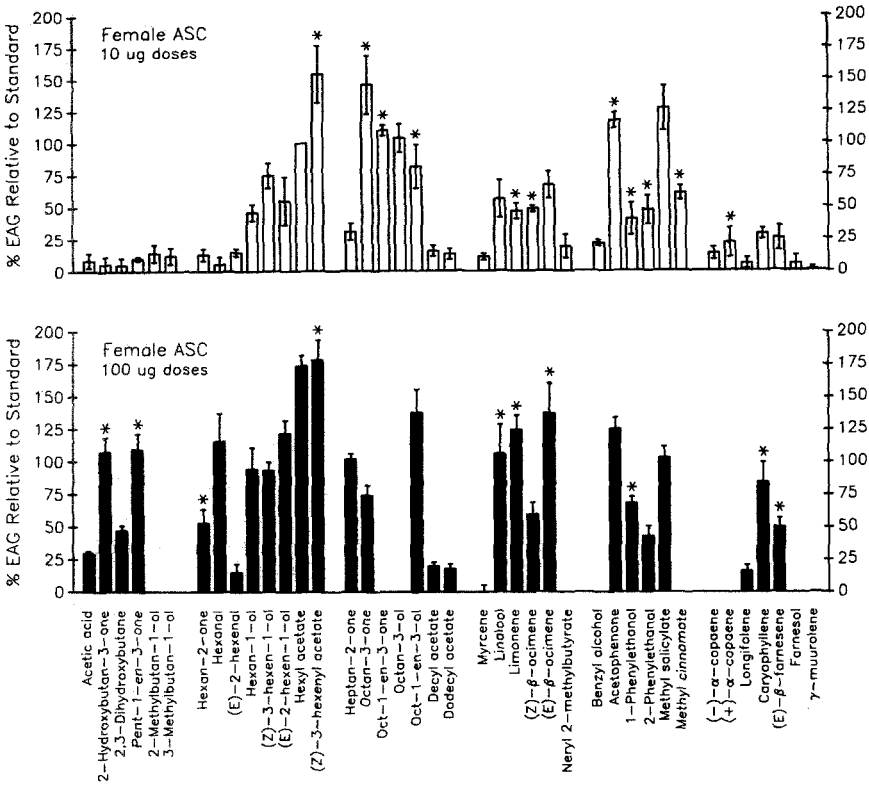


FIG. 1. Mean EAGs (±SEM, $N = 5$) of female alfalfa seed chalcids to alfalfa and red clover volatiles at 10 μg and 100 μg doses. A 100% response is approx. −0.80 mV. Asterisks identify compounds that elicited significantly larger EAGs ($P < 0.05$) from females than males.

then conclude that the less volatile compound is a superior EAG stimulant (e.g., higher transductory affinity of acceptor sites, greater degree and/or numbers of receptors and/or acceptors depolarized or "excited", etc.) than the more volatile compound, because the EAG magnitude per molecule delivered was proportionately far greater.

*Legume Volatiles.* With these analysis limitations considered, EAG recordings for both sexes show $C_6$ acetates, phenolic ketone compounds, and $C_8$ secondary ketones and alcohols (Table 1, Figures 1 and 2) elicited the largest EAGs. The compounds found to elicit the largest EAGs (i.e., >100% of standard) may be ranked as follows: (Z)-3-hexenyl acetate > hexyl acetate ≥ acetophenone ≥ octan-3-one ≥ methyl salicylate > octan-3-ol > oct-1-en-3-one. The compounds that elicited EAGs of moderate magnitudes

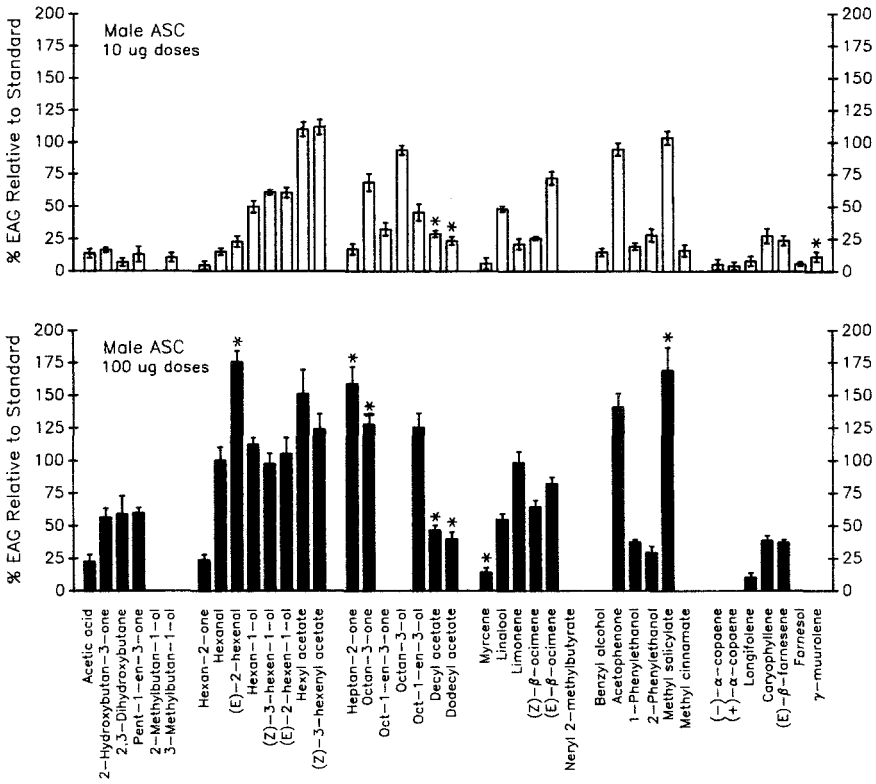


FIG. 2. Mean EAGs (±SEM, $N = 5$) of male alfalfa seed chalcids to alfalfa and red clover volatiles at 10-μg and 100-μg doses. A 100% response is approx. −0.84 mV. Asterisks identify compounds that elicited significantly larger EAGs ($P < 0.05$) from males than females.

(i.e., 50–100% of standard) were: oct-1-en-3-ol ≥ (*E*)-β-ocimene ≥ (Z)-3- and (*E*)-2-hexen-1-ols. These rankings reflect both set-dose experiments (10 and 100 μg) for both females and males (Table 1). However, males varied from females in the order of compound ranking, in that male antennae appear to be relatively less responsive to the $C_8$ secondary ketones and alcohols than to the $C_6$ alcohols (Table 1).

*Sexual Dimorphism.* Analysis of EAG responsiveness to the two set-dose presentations reveals that 58% of the test compounds elicited responses that varied significantly between the sexes (Figures 1 and 2). Further, female antennal responsiveness exceeded that of males for twice the number of volatiles found to elicit sexual dimorphic responses. For the 38 compounds tested in the 10-μg dose experiments, EAG responses by females significantly exceed those

TABLE 2. SENSITIVITY PARAMETERS[a] FOR ANTENNAE OF FEMALE ALFALFA SEED CHALIDS DERIVED FROM DOSE VS. MEAN EAG RESPONSE CURVES (FIGURE 3)

| Compound | Threshold $\log_{10}$ dose ($\mu$g) | Maximum response (% of response to standard) (100-$\mu$g doses) | Dynamic response phase slope [% EAG response/ $\log_{10}$ dose ($\mu$g) step][b] |
|---|---|---|---|
| (Z)-3-Hexen-1-ol | −0.5 | 101.5 | 57.7 |
| Hexyl Acetate | −1.4 | 152.5 | 48.8 |
| (Z)-3-Hexenyl Acetate | −2.2 | 162.1 | 86.1 |
| Octan-3-one | < −3.0 | 140.2 | 77.9 |
| Oct-1-en-3-one | ≤ −4.0 | (> 108.4)[c] | 76.6 |
| Octan-3-ol | −3.2 | (> 104.9) | 82.3 |
| Oct-1-en-3-ol | −1.6 | 134.1 | 78.2:55.8[d] |
| Acetophenone | −2.8 | 138.4 | 65.9 |
| Methyl Salicylate | −2.6 | 117.5 | 59.4 |

[a] See Materials and Methods for definitions of parameters.
[b] The dose-step interval over which slope was determined was the segment between 1-$\mu$g and 10-$\mu$g doses for all but one compound (see [d]).
[c] Percent response values in parentheses were to 10-$\mu$g dose stimulations.
[d] For this compound the slope values are for the dose-step intervals of 0.1 to 1.0 $\mu$g: an average over two $\log_{10}$ steps, 0.1 $\mu$g to 10 $\mu$g.

of males for 15 compounds (four compounds $P < 0.01$ and 11 compounds $P < 0.05$), while male responses exceed those of females for six compounds ($P < 0.05$) (Figures 1 and 2). Similarly, for the 29 compounds tested in the 100-$\mu$g dose experiments, female EAGs exceed those of males for 11 compounds (for seven, $P < 0.01$; remainder, $P < 0.05$) and the opposite was observed for seven compounds (for four, $P < 0.01$; remainder, $P < 0.05$). The compounds that selectively evoked larger EAGs from females than males can be ranked (from highest to lowest degree of sex difference) as: oct-1-en-3-one, octan-3-one, methyl cinnamate, (Z)-3-hexenyl acetate, oct-1-en-3-ol, (Z)- and (E)-β-ocimenes, limonene, acetophenone, caryophyllene, 3-methylbutan-1-ol, pent-1-en-3-one, 1-phenylethanol, hexan-2-one, linalool, 2-phenylethanol, (+)-α-copaene, and (E)-β-farnesene. The rank of compounds that evoked larger EAGs from males than females (but primarily at the higher 100-$\mu$g dose; Table 1) was: (E)-2-hexenal, methyl salicylate, heptan-2-one, octan-3-one, decyl acetate, dodecyl acetate, γ-muurolene, and myrcene.

*Relative Responsiveness of Structural Analogues*

*Positional Isomers.* Antennal responsiveness of female ASCs was greater for the three-than the two-position methylbutanols (100-$\mu$g doses) (Figure 1, Table 1), but for the hexen-1-ols, the (E)-2- geometric isomer elicited larger

EAGs from females than the (*Z*)-3- isomer (100-$\mu$g doses). Both sexes were significantly more responsive to the (*E*)- than the (*Z*)-$\beta$-ocimene geometric isomer or its positional isomer, myrcene. Antennal responsiveness of ASCs was greater for 1-phenylethanol than 2-phenylethanol (100-$\mu$g doses).

*Carbon-Chain-Length Analogs.* Both sexes of ASCs were more responsive to the $C_8$ and $C_7$ saturated ketones than to the $C_6$ analog (Figures 1 and 2, Table 1). Similarly, the $C_8$ monounsaturated ketone elicited significantly greater EAGs than the $C_5$ analog. Hexyl acetate was a more potent EAG stimulant than either decyl or dodecyl acetates. 2-Phenylethanol, with its two-carbon side chain, elicited significantly larger EAGs than benzyl alcohol, with its single-carbon moiety.

*Saturation vs. Unsaturation Analogs.* Antennal responsiveness of females was greater for hexanal than (*E*)-2-hexenal at the 100-$\mu$g dose level, while the opposite was observed for males (Figures 1 and 2, Table 1). Antennal responsiveness was only slightly greater for both the (*E*)-2- and the (*Z*)-3-hexen-1-ols than hexan-1-ol. Only female antennae were significantly more responsive to (*Z*)-3-hexenyl acetate than hexyl acetate. Both sexes were more responsive to the $C_8$ saturated ketone, octan-3-one, than its unsaturated analog, oct-1-en-3-one. Similarly, octan-3-ol elicited significantly larger EAGs than oct-1-en-3-ol but just for males.

*Functional-Group Analogs.* Antennal responsiveness to the four functional-group moieties associated with the saturated and unsaturated six-carbon compounds tested increased in the order of their listing (by generally decreasing volatility) in Table 1. Thus, the least volatile compounds, the $C_6$ acetates, elicited significantly greater EAGs than the increasingly more volatile $C_6$ alcohols, aldehydes, and ketone tested (Figures 1 and 2, Table 1). Antennal responsiveness was greatest for (*Z*)-3-hexenyl acetate followed by (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, and (*E*)-2-hexenal, and similarly was greatest for hexyl acetate followed by hexan-1-ol and hexanal. However, EAGs of females were significantly greater for $C_8$ ketones than for $C_8$ alcohols, while the opposite was observed for males at the 10-$\mu$g dose. Both sexes were significantly more responsive to the ketone, acetophenone, than to its alcohol analog, 1-phenylethanol. Methyl salicylate (with its additional alcohol moiety) elicited significantly larger EAGs than methyl cinnamate (with its two-carbon longer side chain). The hydrocarbon, myrcene, was a far less potent EAG stimulant than its alcohol analog, linalool; conversely, (*E*)-$\beta$-farnesene was a more potent EAG stimulant than its alcohol analog, farnesol.

*Antennal Sensitivity*

*Relative Sensitivity to Legume Volatiles.* Pertinent to the analysis of antennal sensitivity to specific compounds are three parameters (threshold dose, maximum response, and the slope of the dynamic response phase; Table 2) that are

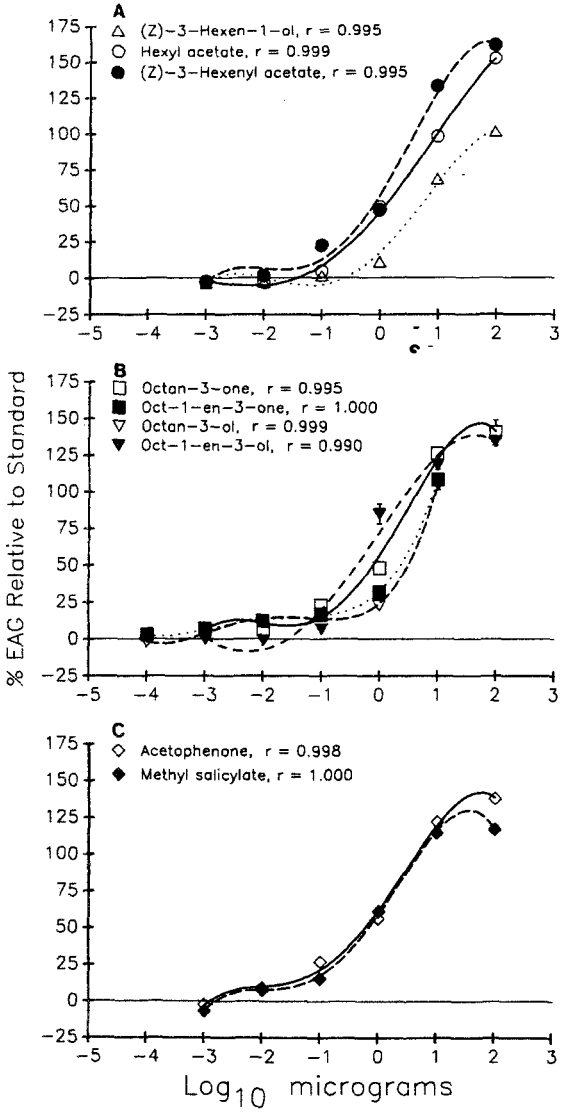


FIG. 3. Dose–EAG curves of female alfalfa seed chalcids to nine compounds found to be potent EAG elicitors: (A) six-carbon alcohol and acetates, (B) eight-carbon ketones and alcohols, and (C) phenolic ketones. Points are means of recordings from five females and vertical lines are ±SEM.

derived from the dose–EAG response curve data (Figure 3). The dose–response curves for the nine compounds tested on female ASC antennae were all sigmoidal (Figure 3), but varied in their threshold, slope, and maximum height values (Table 2). The lowest threshold doses observed were in response to oct-1-en-3-one, octan-3-ol, and octan-3-one. The largest EAG response maxima were to 100 $\mu$g stimulations by (*Z*)-3-hexenyl acetate, hexyl acetate, octan-3-one, and acetophenone. The greatest or steepest slopes of the EAG response curves were attained by doses of (*Z*)-3-hexenyl acetate, octan-3-ol, oct-1-en-3-ol, octan-3-one, oct-1-en-3-one, and acetophenone. Combining the analyses of these three sensitivity parameters, the tested compounds may then be ranked as to female ASC antennal sensitivity in the following order: oct-1-en-3-one $\geq$ octan-3-one $\geq$ (*Z*)-3-hexenyl acetate $\geq$ octan-3-ol $\geq$ oct-1-en-3-ol $\geq$ acetophenone $>$ hexyl acetate $\geq$ methyl salicylate $>$ (*Z*)-3-hexen-1-ol.

*Sensitivity to Six-Carbon Compounds.* Female antennae were more sensitive to (*Z*)-3-hexenyl acetate than the saturated $C_6$ acetate followed by the (*Z*)-3-alcohol, as evidenced by significantly higher mean response values (except at the 1-$\mu$g dose), greater response rates (steeper curve slope), and lower threshold values.

## DISCUSSION

### *Antennal Chemoreceptive Sensitivity*

The EAG is thought to be the summational expression of generator potentials of many simultaneously stimulated receptor cells with potentially different acceptor specificities and/or affinities (Boeckh et al., 1965). The amplitude of the EAG deflection has been interpreted to be a measure of the relative number of acceptors responding to an odor stimulus (Payne, 1975). Because both the position of the electrodes and the presentation of the odor stimuli spanned the entire length of the antennae, the EAGs recorded should reflect the overall population size of chemoreceptors and/or acceptors present. Thus, the EAG results reported here for alfalfa seed chalcids suggest significant differences in size of antennal receptor and/or acceptor populations for the various alfalfa host-plant and nonhost-plant (red clover) volatiles examined. In general, both female and male ASCs possess antennal chemoreceptive sensitivities that were keen, dynamic, and selective to certain specific compounds and to certain classes of compounds, defined by their carbon-chain length, structure, isomerism, and/or functional group (Table 1). The antennae of ASCs are stimulated to a greater extent and thus possess larger populations of responsive receptors and/or acceptors for constituents of three (of the seven) structural classes of compounds investigated: (1) the six-carbon acetates, primary alcohols, and aldehydes; (2)

the eight-carbon, three-position ketones and secondary alcohols; and (3) the aromatic methyl ketones and methyl esters.

*ASC Sexual Dimorphism.* There appear to be significant differences between the ASC sexes in the size of their receptor and/or acceptor populations responsive to a majority (58% overall) of the alfalfa and legume volatiles tested. Overall, the receptivity of female ASCs significantly exceeded that of males for 18 of the 38 tested compounds, while the reverse was observed for just eight of the 38 compounds (Figures 1 and 2, Table 1). Furthermore, the dominance of female receptivity to legume volatiles over that of males is evident for: (1) six of the top 10 EAG stimulants (for males > females, 1/10) (Figure 3), (2) six of the 14 demonstrated behavioral attractants (for males > females, 3/14) (Table 1), and (3) four of the six demonstrated ovipositional stimulants (for males > females, 1/6) (Table 1).

The observed sexual dimorphism in antennal receptivity to volatiles of host- and nonhost-plant odors might be a consequence of either (1) chromosomal genetics or (2) natural selection. First, because male–female sex determination in Hymenoptera is due to a haploid–diploid chromosomal endowment, the apparent sexual dimorphism in antennal receptivity to volatiles of host- and nonhost-plant odors might be an expression of the different genotypes of the sexes, potentially creating differences in numbers of receptors. We suggest the second possibility, that this dimorphism in reception reflects differences between the sexes in perception of these compounds that has evolved through the selective advantage of the use of these olfactory cues in the survival requisites of host finding, food foraging, and oviposition.

Both sexes share some of these survival requisites and roles that are reflected in some common searching and foraging behaviors. Because ASCs are monophagous, host finding is selective and critical; however, they forage for nectar on host and nonhost plants. The primarily floral volatiles from alfalfa are: (*E*)-β-ocimene, 3-methylbutanol, decyl and dodecyl acetates, and methyl salicylate; those from red clover are: acetophenone, methyl cinnamate, (*E*)-2-hexenal, 2-hydroxybutan-3-one, and 2,3-dihydroxybutane (Table 1). None of these alfalfa floral volatiles were attractive or stimulatory to females in laboratory behavioral bioassays (Kamm and Buttery, 1983, 1986a,b). Because methyl salicylate was a potent elicitor of EAGs, we suspect it could have an impact on behavior but was overlooked in previous bioassays (Kamm and Buttery, 1983, 1986a,b).

The obvious specialization and diversification between the sexes is in their reproductive physiology and behavior. Since mating and oviposition of ASCs are restricted to their alfalfa host plant, one might assume that alfalfa odors that are indicative of the phenological quality or suitability of pods and/or seeds for exploitation might influence the selection by female ASCs of not just oviposition sites (Kamm and Buttery, 1986b; Kamm, 1989) but also calling and/or mating sites and/or release of pheromone (Riddiford and Williams, 1967; Riddiford,

1967; Raina, 1988). Similarly, because females call and release pheromone in the immediate context of host kairomones, the perception of these two distinct semiochemical odors may have coevolved in chemical communication to the degree that alfalfa odors might influence, enhance, or synergize the attractiveness of the female-released pheromone to orienting males. Such enhancement or synergism of the attractiveness of pheromones by plant volatiles has been observed for a number of insect species (e.g., Dickens et al., 1990; Light, unpublished).

*Green Leaf Volatiles.* The saturated and unsaturated [(*E*)-2- and (*Z*)-3-] $C_6$ acetates, alcohols, and aldehydes are the most common class of botanical volatiles, having been termed the general "green leaf volatiles" (GLVs). The GLVs are ubiquitous in the plant kingdom and are produced from the general oxidative breakdown of simple fatty acids (Buttery, 1981; Tressel et al., 1981; Schreier, 1984; Visser et al., 1979). Plant species and their plant parts differ in composition (occurrence and ratio) of these GLV compounds (Visser et al., 1979; Buttery, 1981; Van Straten and Maarse, 1983). (*Z*)-3-Hexenyl acetate and (*Z*)-3-hexen-1-ol are the dominant contributors to the odors of many forage legumes, especially alfalfa (Kami, 1978, 1983; Buttery and Kamm, 1980; Buttery et al., 1982, 1984; Srinivas, 1986) (Table 1). These two particular GLVs were potent EAG stimulants and were moderate to low in potency in eliciting behavioral responses (Figure 4), and they should be included in future behavioral tests with various chalcid species.

Because the GLVs, in particular the $C_6$ acetates, were found to be very potent EAG stimulants, we conclude that ASC antennae possess large populations of receptor and/or acceptors responsive to these compounds. Furthermore, this substantial olfactory receptor and/or acceptor endowment suggests a selective detection and perception of the GLVs by ASCs. Similarly, the GLVs have been found to be potent EAG and receptor cell stimulants for a great number of diverse insects (see Light et al., 1988; Visser, 1983, 1986), e.g. *Ceratitis capitata* (Light et al., 1988), *Dacus dorsalis* (Light and Jang, 1987), *Anthonomus grandis* (Dickens, 1984), *Leptinotarsa decemlineata* (Visser, 1979), and *Trirhabda bacharides* (Dickens and Boldt, 1985).

*Antennal Responsiveness of Other Hymenoptera.* Half the legume compounds tested here have also been the subject of electrophysiological investigations on other species of Hymenoptera from diverse families and resource ecologies—from ichneumonid parasitoids, nyssonine wasps, horntail wood wasps, to eusocial hymenopteran ants and bees. Many of these compounds have been tested on the eusocial Hymenoptera because of their importance to chemical communication, having been identified as pheromones or constituents and/or products of various glands (anal, cephalic, Dufour's, mandibular, poison, and/or sting) (Wheeler and Duffield, 1988). Single-cell electrophysiological recordings on both an ant species, *Lasius fuliginosus* (Dumpert, 1972), and *Apis*

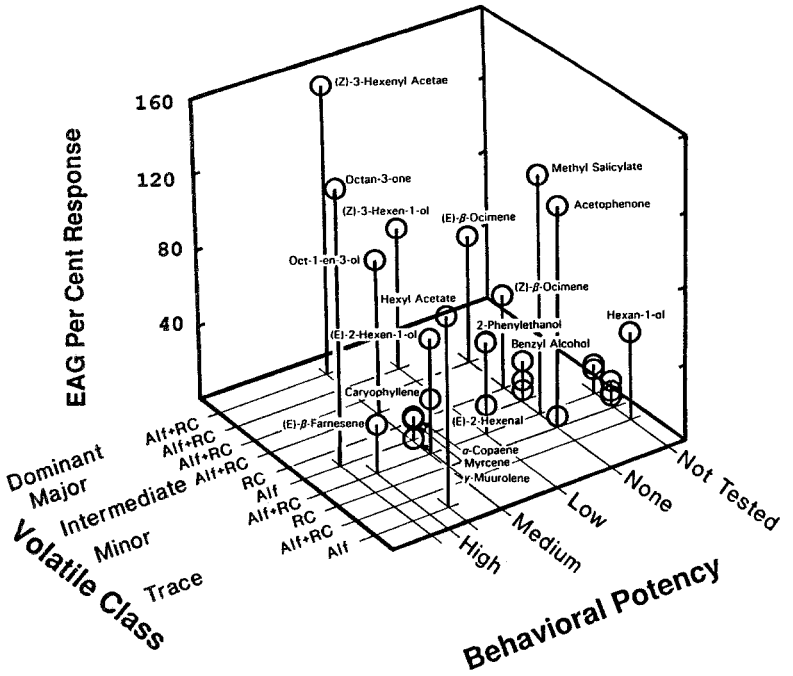


FIG. 4. Relationship of the tested volatiles to their: evoked EAGs by female ASCs, behavioral potency classification for female ASCs, and contribution classification to the odor composition of alfalfa, red clover, or both legumes. The EAG data are the mean percent response relative to standard for 10-$\mu$g doses of volatiles. Behavioral potency classification is based on threshold doses (TD): "high" potency (TD $\geq$ 0.025 $\mu$g), "medium" potency (TD $\geq$ 2.5 $\mu$g), and "low" potency (TD $>$ 25.0 $\mu$g). Contribution classification or "volatile class" for the odor constituents of leaves and/or pods is delineated as: "dominant" ($>$30% of odor), "major" ($>$10%), "intermediate" ($>$5%), "minor" ($>$1%), and "trace" ($<$1%). " Alf," "RC," and "RC+Alf" designate the presence of those volatiles in alfalfa, red clover, or both odors.

*mellifera* (Lacher, 1964; Vareschi, 1971) have established that the sensitivity to many of these compounds is based on the antennae being endowed with an array of different "generalist" cell types, each possessing a rather narrow specificity for certain structural, chain-length, and functional-group classes of compounds. The presence of an array of generalist cell types might also occur for ASCs.

*Plant Odor Composition, Chemoreceptivity, and Behavior*

No correlations were found between either the specific occurrence and concentration of a plant volatile and ASC behavior (Table 1 and Figure 4). ASCs were highly receptive to the dominant odorants of alfalfa, (Z)-3-hexenyl

acetate and (*Z*)-3-hexen-1-ol, but these compounds individually elicited only medium and low potency (respectively) overall behavioral responses (low attractancy, but moderate arrestment and ovipositional stimulation) (Table 1 and Figure 4). However, the three compounds that were most potent behaviorally (volatiles that elicited significant levels of behavior at the lowest threshold doses) were merely minor or trace odor constituents of either alfalfa or red clover. For example, octan-3-one and hexyl acetate are minor and trace components of alfalfa odor, but they are potent elicitors of both EAG responses and behavioral attraction, arrestment, and oviposition responses. In contrast, (*E*)-$\beta$-farnesene is a minor to trace component of both alfalfa and red clover odors and low in EAG potency, but it is second overall to octan-3-one as a very potent elicitor of ASC behavioral attraction and a potent elicitor of oviposition.

Nearly all of the plant components that were behaviorally active elicited moderate [e.g., (*E*)-2- and (*Z*)-3-hexen-1-ols and oct-1-en-3-ol] to potent [e.g., (*Z*)-3-hexenyl acetate, octan-3-one and hexyl acetate] EAGs. Three exceptions are minor constituents of alfalfa odors that are relatively impotent as EAG stimulants. Two of these volatiles, $\alpha$-copaene and $\gamma$-muurolene, are minor components of alfalfa pod odor and are moderately potent in eliciting close-range flight and pedestrian orientation, while $\gamma$-muurolene inhibits oviposition (Table 1) (Kamm and Buttery, 1983, 1986b). Myrcene (a minor component of both alfalfa flower and leaf odors) also elicited a very low EAG response but was a very potent stimulant for oviposition but not flight. These behaviors were observed only when females were in contact with the host (or an evaporative substrate, i.e., filter paper) (Kamm and Buttery, 1983, 1986b). Considering both the low antennal olfactory responses and the low volatility of these compounds, female ASCs may detect these semiochemicals with contact chemoreceptors on the tarsi, ovipositor, or tip of antennae (Kamm and Buttery, 1986b). Chalcids constantly tap plant surfaces with the tips of their antennae in a manner suggesting both a chemo- and mechanoreceptive evaluation of substrates.

The detection and recognition of host from nonhost plant is crucial for seed chalcids, and thus both the presence of host species-specific volatiles and their selected activity as kairomones and allomones is expected. The seed chalcids might follow sequential perceptual assessments and behavioral responses to olfactory information in their immediate environment. The activation of flight orientation by ASCs might be elicited by general volatiles of clover-related legume species, but the maintenance of the anemotaxis (i.e., the flight to or arrival on the target plant) might require the discrimination of alfalfa-specific volatiles. Moreover, ASCs might be initially attracted to legume kairomones, but this attraction might be repelled (inhibited or interrupted) by allomonal nonhost volatiles. However, we doubt that nonhost components can confound host finding because chalcids readily identify their hosts from nonhosts in mixed stands in nature. Finally, close-range orientation and alightment might be elicited by alfalfa volatiles followed by contact host-assessment and either stimu-

lation or inhibition and/or interruption of oviposition. The odorants in common between alfalfa and red clover can be segregated into four classes based on behavioral response of female ASCs: (1) volatiles that elicited no responses [(*E*)-β-ocimene and methyl salicylate], (2) volatiles that elicited only flight orientations (attraction) [oct-1-en-3-ol and (*E*)-2-hexenal], (3) volatiles that elicited flight and pedestrian orientations [(*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol], and (4) volatiles that elicited both of these orientations and oviposition [(*Z*)-3-hexenyl acetate, (*E*)-β-farnesene and caryophyllene] (Table 1, Figure 4). Some of these compounds common to both legumes, for which ASCs have a keen chemoreceptivity and which elicit orientation behavior, are believed to be involved in initial host (or legume) finding; then the alfalfa-specific volatiles with demonstrated behavioral impact [hexyl acetate, γ-muurolene, α-copaene, and myrcene (Table 1)] are perhaps close-range cues that females use for host identification and/or discrimination. Conversely, ASC antennae were keenly sensitive to acetophenone (a red clover-specific volatile) and thus it might be a distinguishing cue for identification of nonhost legumes. Without sensitivity to such nonhost compounds, ASC females could expend considerable time and energy maladaptively responding to compounds present in both legumes. Appropriate flight bioassays are now required to identify the specific behaviors (initiation of flight, orientation, landing, and assessment of the source) elicited by specific host and nonhost volatiles when presented individually and in various binary and multicomponent combinations, as was accomplished using plant parts (Kamm, 1989). Information herein will provide insight into how chalcids perceive and use olfactory cues to exploit their host plant.

*Acknowledgments*—Special thanks to Mr. W.E. Gavin for rearing the insects and conducting the electroantennograms. We thank Drs. Judith Eash and Bruce Campbell for valuable comments on the manuscript.

## REFERENCES

BOECKH, J., KAISSLING, K.-E., and SCHNEIDER, D. 1965. Insect olfactory receptors. *Cold Springs Harbor Symp. Quant. Biol.* 30:263–280.

BUTTERY, R.G. 1981. Vegetable and fruit flavors, pp. 175–216, *in* R. Teranishi, R.A. Flath, and H. Sugisawa (eds.). Flavor Research, Recent Advances. Marcel Dekker, New York.

BUTTERY, R.G., and KAMM, J.A. 1980. Volatile components of alfalfa: Possible insect host plant attractants. *J. Agric. Food Chem.* 28:978–981.

BUTTERY, R.G., KAMM, J.A., and LING, L.C. 1982. Volatile components of alfalfa flowers and pods. *J. Agric. Food Chem.* 30:739–742.

BUTTERY, R.G., KAMM, J.A., and LING, L.C. 1984. Volatile components of red clover leaves, flowers and seed pods: Possible insect attractants. *J. Agric. Food Chem.* 32:254–256.

DICKENS, J.C. 1984. Olfaction in the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae): Electroantennogram studies. *J. Chem. Ecol.* 10:1759–1785.

DICKENS, J.C., and BOLDT, P.E. 1985. Electroantennogram responses of *Trirhabda bacharides* (Weber) (Coleoptera: Chrysomelidae) to plant volatiles. *J. Chem. Ecol.* 11:767–779.

DICKENS, J.C., JANG, E.B., LIGHT, D.M., and ALFORD, A.R., 1990. Enhancement of insect pheromone responses by green leaf volatiles. *Naturwissenschaften* 77:29–31.

DUMPERT, K. 1972. Alarmstoffrezeptoren auf der Antenne von *Lasius fuliginosus* (Latr.) (Hymenoptera, Formicidae). *Z. Vergl. Physiol.* 76:403–425.

KAMI, T. 1978. Qualitative and quantitative analyses of the essential oils of red and Ladino white clovers. *J. Agric. Food Chem.* 26:1194–1197.

KAMI, T. 1983. Composition of the essential oil of alfalfa. *J. Agric. Food Chem.* 31:38–41.

KAMM, J.A. 1989. In-flight assessment of host and nonhost odors by alfalfa seed chalcid (Hymenoptera: Eurytomidae). *Environ. Entomol.* 18:56–60.

KAMM, J.A., and BUTTERY, R.G. 1983. Response of the alfalfa seed chalcid, *Bruchophugus roddi*, to alfalfa volatiles. *Entomol. Exp. Appl.* 33:129–134.

KAMM, J.A., and BUTTERY, R.G. 1986a. Response of the alfalfa and clover seed chalcids (Hymenoptera: Eurytomidae) to host plant components. *Environ. Entomol.* 15:1244–1249.

KAMM, J.A., and BUTTERY, R.G. 1986b. Ovipositional behavior of the alfalfa seed chalcid (Hymenoptera: Eurytomidae) in response to volatile components of alfalfa. *Environ. Entomol.* 15:388–391.

LACHER, V. 1964. Elektrophysiologische Untersuchungen an Einzelnen Rezeptoren fur Geruch, Kohlendioxyd, Luftfeuchtigkeit und Temperatur auf den Antennen der Arbeitsbiene und der Drohne. *Z. Vergl. Physiol.* 48:587–623.

LIGHT, D.M. 1983. Sensitivity of antennae of male and female *Ips paraconfusus* (Coleoptera: Scolytidae) to their natural aggregation pheromone and its enantiomeric components. *J. Chem. Ecol.* 9:561–584.

LIGHT, D.M., and JANG, E.B. 1987. Electroantennogram responses of the oriental fruit fly, *Dacus dorsalis*, to a spectrum of alcohol and aldehyde plant volatiles. *Entomol. Exp. Appl.* 45:55–64.

LIGHT, D.M., JANG, E.B., and DICKENS, J.C. 1988. Electroantennogram responses of the Mediterranean fruit fly, *Ceratitis capitata*, to a spectrum of plant volatiles. *J. Chem. Ecol.* 14:159–180.

PAYNE, T.L. 1975. Bark beetle olfaction. III. Antennal olfactory responsiveness of *Dendroctonus frontalis* Zimmerman and *D. brevicomis* Le Conte (Coleoptera: Scolytidae) to aggregation pheromones and host tree terpene hydrocarbons. *J. Chem. Ecol.* 1:233–242.

RAINA, A.K. 1988. Selected factors influencing neurohormonal regulation of sex pheromone production in *Heliothis* species. *J. Chem. Ecol.* 14:2063–2069.

RIDDIFORD, L.M. 1967. *trans*-2-Hexenal: Mating stimulant for polyphemus moth. *Science* 158:139–141.

RIDDIFORD, L.M., and WILLIAMS, C.M. 1967. Volatile principle from oak leaves role in sex life of the polyphemus moth. *Science* 155:589–590.

SCHREIER, P. 1984. Chromatographic Studies of Biogenesis of Plant Volatiles. A. Huthig Verlag, New York.

SRINIVAS, S.R. 1986. Volatile constituents in alfalfa and red clover extracts, pp. 343–353, *in* B.M. Lawrence, B.D. Mookherjee, and B.J. Willis (eds.). Flavors and Fragrances: A World Perspective. Proceedings, 10th International Congress of Essential Oils, Washington, D.C.

STRONG, F.E. 1962. Laboratory studies of the biology of the alfalfa seed chalcid, *Bruchophugus roddi*, Guss. (Hymenoptera: Eurytomidae). *Hilgardia* 32:229–249.

TRESSEL, R., BAHRI, D., and ENGEL, K.-H. 1981. Lipid oxidation in fruit and vegetables, pp. 213–232, *in* R. Teranishi and H. Barrera-Benitez (eds.). Quality of Selected Fruits and Vegetables of North America. ACS Symposium 170. American Chemical Society, Washington, D.C.

VAN STRATEN, S., and MAARSE, H. 1983. Volatile Compounds in Food, 5th ed. Central Institute for Nutrition and Food Research TNO, Zeist, The Netherlands.

VARESCHI, E. 1971. Duftunterscheidung bei der Honigbiene-Einzelzell-Arbeitung und Verhaltensreaktionen. *Z. Vergl. Physiol.* 75:143–173.

VISSER, J.H. 1979. Electroantennogram responses of the Colorado beetle, *Leptinotarsa decemlineata* to plant volatiles. *Entomol. Exp. Appl.* 25:86–97.

VISSER, J.H. 1983. Differential sensory perceptions of plant compounds by insects. *Am. Chem. Soc. Symp. Ser.* 208:215–230.

VISSER, J.H. 1986. Host odor perception in phytophagous insects. *Annu. Rev. Entomol.* 31:121–144.

VISSER, J.H., VAN STRATEN, S., and MAARSE, H. 1979. Isolation and identification of volatiles in the foliage of potato, *Solanum tuberosum*, a host plant of the Colorado beetle, *Leptinotarsa decemlineata*. *J. Chem. Ecol.* 5:13–25.

WHEELER, J.W., and DUFFIELD, R.M. 1988. Pheromones of Hymenoptera and Isoptera, pp. 59–206, *in* E.D. Morgan and N.B. Mandava (eds.). CRC Handbook of Natural Pesticides, Vol IV, Pheromones, Part B. CRC Press, Boca Raton, Florida.